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RESEARCH PRODUCTS

Alzheimer's Research Discovery Pack — Cat# DSV05

BACKGROUND:

Alzheimer's disease is histopathologically characterized by the presence of intracellular neurofibrillary tangles and extracellular amyloid plaques. Neurofibrillary tangles consist of paired helical filaments of the hyperphosphorylated microtubule-associated protein tau (1), while plaques are predominantly comprised of insoluble deposits of the β-amyloid peptide, a 39-43 amino acid peptide derived from proteolytic cleavage of the β-amyloid precursor protein (βAPP) (2). Mutations in several gene products, including presenilin-1, presenilin-2, and the β-amyloid precursor protein are associated with early onset Alzheimer's disease, resulting in increased extracellular concentrations of the longer form of the β-amyloid peptide Aβ 1-42 relative to Aβ 1-40 (3,4,5). It is this longer form of Aβ which has been shown to be toxic to neurons, and may serve as a catalysts for the aggregation and deposition of Aβ to produce the neurotoxic effects associated with senile plaque formation. Using the Aβ peptide in a yeast two-hybrid screen, a novel interacting protein designated the endoplasmic reticulum-associated amyloid beta-peptide-binding protein (ERAB)/L-3-hydroxyacyl-CoA dehydrogenase type II was identified, and shown to be expressed at high levels in Alzheimer's disease-affected brain (6,7). Interestingly, the neurotoxic effects observed with Aβ were shown to be attenuated by expression levels of ERAB, suggesting that ERAB may contribute to neuronal dysfunction in Alzheimer's disease (6).

KIT CONTENTS AND CHARACTERISTICS:

Antibody	Cat#	Clone	Isotype	Epitope	Applications
Presenilin-1 [31-46] (Ab-1)	Cat# PC244	n /a	Rabbit polyclonal IgG	Amino acids 31-46 N-terminus of the mouse presenilin 1 sequence	IF
Presenilin-1 [303-316] (Ab-2)	Cat# PC267	n /a	Rabbit polyclonal IgG	Amino acids 303-316 from the loop region of the mouse presenilin 1	WB, IF
Presenilin-2 (Ab-1)	Cat# PC305	n /a	Rabbit polyclonal IgG	Amino acids 7-24 of the human presenilin-2 protein	WB, FS
Presenilin-2 (Ab-2)	Cat# PC235	n /a	Rabbit polyclonal IgG	Amino acids 324-335 of the human presenilin-2	WB, FS
ERAB (Ab-1)	Cat# PC243	n /a	Rabbit polyclonal IgG	Amino acids 100-116 of the human ERAB protein	WB, PS
β-Amyloid [1-40] (Ab-1)	Cat# PC149	n /a	Rabbit polyclonal IgG	Not determined	DB, ELISA, WB
β-Amyloid [1-42] (Ab-1)	Cat# PC150	n /a	Rabbit polyclonal IgG	Not determined	DB, ELISA, WB
Anti-APP	Cat# 171537	3E9	IgG	Amino acids 18-38 of human APP_{695}	WB, IHC

Legend: WB=Western Blot, IF=Immunofluorescence, FS=Frozen Sections, PS=Paraffin Sections, IHC=Immunohistochemistry, DB=Dot Blot

HOW SUPPLIED:

Exhibit 9

PC244, PC267, PC305, PC235, PC243, contain 10 µg polyclonal purified rabbit IgG in 0.1 ml of of 0.05 M sodium phosphate buffer containing 50% glycerol. 171537 contain 10µg polyclonal purified rabbit IgG in 0.05 ml of of 0.05 M sodium phosphate buffer containing 50% glycerol PC149, PC150 contain 10 µg of affinity purified antibody in PBS.

STORAGE:

PC244, PC267, PC305, PC235, PC243: Store at -20°C. For long term storage, aliquot into smaller volumes and store at -20°C. Avoid multiple freeze/thaw cycles. If stored under proper conditions, product guaranteed until expiration date stated.

ORIGIN & CHARACTERISTICS:

Presenilin-1 [31-46] (Ab-1), Cat# PC244: Presenilin 1 [31-46] (Ab-1) is a rabbit polyclonal antibody generated by repeated immunization of rabbits with a synthetic peptide corresponding to amino acids 31-46 (DSQERQQQHDRQRLDN) from the N-terminus of the mouse presenilin 1 sequence. For immunofluorescence on dissociated cultured rat hippocampal neurons, this antibody was diluted 1:25 in blocking buffer and incubated with cells for 2 hours at 37°C. The antibody was tested for specificity by immunoblot analysis of neuronal cell lysates, and by preincubation of the antiserum with the peptide immunogen. A prominent band of 98 kDa, believed to be a PS-1 dimer, was detected. In addition, this antibody detected a 30 kDa band, a band believed to represent an endoproteolytic fragment of Presenilin 1. Antibodies should be titrated for optimal results in individual systems.

Presenilin-1 [303-316] (Ab-2), Cat# PC267: Presenilin 1 [303-316] (Ab-2) is a rabbit polyclonal antibody generated by repeated immunization of rabbits with a synthetic peptide corresponding to amino acids 303-316 (DPEAQRRVPKNPKY) from the loop region of the mouse presenilin 1 sequence. Presenilin 1 [303-316] (Ab-2) at a dilution of 1:2,000 was tested for specificity by western blot analysis using neuronal cell lysates. A prominent band at approximately 98 kDa was detected, and is believed to correspond to a PS-1 dimer. A smaller 21 kDa endoproteolytic fragment was also detected. Preabsorption of Presenilin 1 [303-316] (Ab-2) with the peptide immunogen completely abolished labeling. For immunofluorescence on dissociated cultured rat hippocampal neurons, this antibody was diluted 1:25 in blocking buffer and incubated with cells for 2 hours at 37°C. Antibodies should be titrated for optimal results in individual systems.

Presenilin-2 (Ab-1), Cat# PC305: Presenilin-2 (Ab-1) was generated by immunizing rabbits with a synthetic peptide corresponding to amino acids 7-24 from the N-terminus of the human presenilin-2 protein (SDSEEEVCDERTSLMSAE). This amino acid sequence is identical to presenilin-2 from mouse and rat. Presenilin-2 (Ab-1) recognizes an N-terminal proteolytic fragment at 28 kDa in western blots using mouse or rat brain tissue extract, as well as in HeLa cell extract. Additional bands are also observed which likely correspond to aggregated, modified, or intact PS-2 depending upon extraction conditions. Immunohistochemistry was performed using floating mouse brain sections fixed in 4% paraformaldehyde. Antibodies should be titrated for optimal results in individual systems. Suggested starting concentration for western blot is 1-2 µg/ml, and 0.5-2 µg/ml for frozen sections.

Presenilin-2 (Ab-2), Cat# PC235: Presenilin-2 (Ab-2) was generated by immunizing rabbits with a synthetic peptide corresponding to amino acids 324-335 from the C-terminus of the human presenilin-2 protein (EEDSYDSFGEPS). This amino acid sequence is identical to presenilin-2 from mouse and rat. Presenilin-2 (Ab-2) recognizes a C-terminal proteolytic fragment at 20 kDa in western blots using mouse or rat brain tissue extract, as well as in HeLa cell extract. Additional bands are also observed which likely correspond to aggregated, modified, or intact PS-2 depending upon extraction conditions. Immunohistochemistry was performed using floating mouse brain sections fixed in 4% paraformaldehyde. Antibodies should be titrated for optimal results in individual systems. Suggested starting concentration for western blot is 1-2 µg/ml, and 2 µg/ml for frozen sections.

ERAB (Ab-1), Cat# PC243: ERAB (Ab-1) was generated by immunizing rabbits with a synthetic peptide corresponding to amino acids 100-116 (TYNLKKGQTHTLEDFQR) from the human ERAB protein. This peptide was conjugated to KLH via an N-terminal cysteine. By western blot, ERAB (Ab-1) detects native protein migrating at approximately 27 kDa in SDS-PAGE blots using total cell extract from the human SK-N-SH neuroblastoma cell line. This antibody does not detect ERAB in mouse or rat brain tissue extract. Immunohistochemistry was performed on

paraffin embedded tissue from human cerebellum. Staining was completely abolished by preincubating the affinity purified antibody with control peptide at 10^{-6}M. Unconjugated ERAB Control Peptide has been included for use as an internal control for antibody specificity. Antibodies should be titrated for optimal results in individual systems. Suggested starting concentration for western blot is 1-3 µg/ml, and 1-2 µg/ml for immunohistochemistry.

β-Amyloid [1-40] (Ab-1), Cat# PC149: β-Amyloid [1-40] (Ab-1) is a rabbit polyclonal IgG against a C-terminal fragment of β-amyloid peptide [1-40] which has been preabsorbed against both the full length [1-42] and [1-43] followed by affinity purification using a C-terminal fragment of Aβ [1-40]. This antibody is specific for β-amyloid peptide [1-40] and shows no reactivity to β-amyloid peptide [1-42] or β-amyloid peptide [1-43]. This antibody is recommended for use in the quantitative determination of β-amyloid peptide [1-40] (7,8). Suggested working concentration is 0.1-0.5 µg/mL for western blot, dot blot and ELISA. The sensitivity level for sandwich ELISA is 5-15 pg. For RIA, the suggested working concentration is 0.5 µg/mL. Antibodies should be titrated for optimal results in individual systems.

β-Amyloid [1-42] (Ab-1), Cat# PC150: β-Amyloid [1-42] (Ab-1) is a rabbit polyclonal IgG against a C-terminal fragment of β-amyloid peptide [1-42] which has been preabsorbed against both the full length [1-40] and [1-43] followed by affinity purification using a C-terminal fragment of Aβ [1-42]. This antibody is specific for β-amyloid peptide [1-42] and shows no reactivity to β-amyloid peptide [1-40] or β-amyloid peptide [1-43]. This antibody is recommended for use in the quantitative determination of β-amyloid peptide [1-42] (7,8). Suggested working concentration is 0.1-0.5 µg/mL for western blot, dot blot and ELISA. The sensitivity level for sandwich ELISA is 15-25 pg. For RIA, the suggested working concentration is 0.5 µg/mL. Antibodies should be titrated for optimal results in individual systems.

Anti-APP, Cat# 171537: Anti-APP is a mouse monoclonal antibody generated by immunizing with a synthetic peptide (LEVPTDGNAGLLAEPQIAMFC) corresponding to residues 18-38 of human APP_{695}. Amyloid β protein is the major protein subunit of amyloid fibrils and deposits characteristics of the neurodegenerative disorder Alzheimer's disease. This monoclanl anti-APP antibody is specific for human APP. It is supplied as purified IgG, prepared from mouse ascties by ammonium sulfate precipiation and protein A affinity chromatography. This antibody is suitable for immunoblotting and for staining formalin fixed parafin sections. Variables associated with assay conditions will dictate the proper working dilutions.

COMMENTS:

Suggested starting concentrations are provided; antibody should be titrated for optimal results in individual sample types.

REFERENCES:

1. Trojanowski, J.Q. and Lee, V.M.-Y. 1995. FASEB J. 9: 1570-1576.

2. Tanzi, R., et al. 1996. Neurobiol. Dis. 3: 159-168.
3. Borchelt, D.R., et al. 1996. Neuron 17: 1005-1013.
4. Duff, K., et al. 1996. Nature 383: 710-713.
5. Citron, M., et al. 1997. Nature Med. 3: 67-72.
6. Yan, S.D., et al. 1998. Nature 389: 689-695.
7. He, X.Y., et al. 1998. J. Biol. Chem. 273: 10741-10746.

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ONCOGENE RESEARCH PRODUCTS
650 Albany Street
Boston, MA 02118
Tel. (617) 536-2223 (800) 662-2616
Fax (617) 536-9911 (800) 828-4871
E-mail: oncogene@apoptosis.com

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